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Source and Portal of Entry of Bacteria Found in Bruised Poultry Tissue[1]

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ABSTRACT

HAMDY, M. K. (University of Georgia, Athens), N. D. BARTON, AND W. E. BROWN. Source and portal of entry of bacteria found in bruised poultry tissue. Appl. Microbiol. **12**:464-469. 1964.—Bacteriological studies revealed that normal tissue, air sacs, feathers, skin of birds, poultry feed, gut, and chicken droppings were sources of the predominant organisms, including staphylococci, found in bruised poultry tissue. Further investigation of normal tissue revealed that after the intramuscular injection of *Staphylococcus aureus*, marker strain (MS), the organism was eliminated from these tissues within 7 days. However, when these tissues were traumatized 3 days after injection, the number of the test organism increased, and the organism was present on the 7th day after inoculation. Poultry feed and fecal material contained a large number of staphylococci identical to those isolated from bruised tissue (McCarthy, Brown, and Hamdy, 1963), thereby implicating the gut as a possible portal of entry. When a pathogenic marker strain of *S. aureus* was established in the intestinal tract of chickens by administering an active culture of this organism either in their drinking water or by gavage, it was recovered from the traumatized tissue. The incidence of positive culture of *S. aureus* MS in these tissues correlated with age of bruise, reaching 22 to 33% immediately after contusion and at the early stages of healing (1 to 3 days post bruise) and decreasing thereafter from 11 to 0% on the 4th through 6th days after bruise infliction. The air sac was also found to be a site by which bacteria may enter the traumatized tissues, but to a limited extent.

There is now substantial evidence that a few bacteria may be present in living tissue. Much of this evidence incriminates the intraintestinal bacteria which are absorbed from the intestinal lumen into the circulating blood in normal as well as shocked animals. Lepovetsky, Weiser, and Deatherage (1953) reported that, although a majority of the popliteal and prescapular lymph nodes of cattle contained gram-negative rods, gram-positive cocci, diphtheroids, and several types of anaerobes, only a few samples of the bone marrow and muscle tissue appeared to harbor bacteria. Gunderson, McFadden, and Kyle (1954) isolated organisms of the genera *Salmonella* and *Staphylococcus* from equipment that was directly or indirectly contaminated by intestinal or visceral contents. Jacob et al. (1954) established the presence of gram-negative bacteria in the liver and other tissues of dogs, and showed that these organisms were rarely found in the normal tissues immediately after death, whereas gram-positive rods and cocci were found frequently. They also established that, when the dogs were subjected to hemorrhagic shock for 6 hr and examined immediately after death, there was a sharp increase in the incidence of both gram-negative and gram-positive bacteria. Frank et al. (1961) stated that this exposure to hemorrhagic shock so weakens the antibacterial defenses that both gram-positive and -negative organisms could be grown out more readily than in normal animals. Ayres (1955) stated that microorganisms, which gain entrance into tissues, penetrate the intestinal wall and are carried to various parts of the body by the blood. Fine, Rutenburg, and Schweinburg (1959) established that bacteria regularly traverse the intestinal barrier and that, even though they multiply when the antibacterial defenses are down, their number in the first filtering depots (liver and mesenteric lymph nodes) is too small to contribute significantly to a general endotoxemia. Gordon et al. (1955) and Benacerraf (1960) demonstrated that the intestinal mucosa of the normal mouse is not impermeable to bacteria. The former authors also showed that *Serratia marcescens*, established in the alimentary tract by oral administration, was more or less regularly migrating through the normal intestinal mucosa in small numbers and reaching the mesenteric lymph nodes where it was trapped. After X irradiation with 700 r (1 electrostatic unit per cubic centimeter of standard air or 2.083×10^9 ion pairs per cubic centimeter of standard air), the incidence of positive cultures for this organism showed that the liver or spleen was infected before the blood stream was invaded.

Payne and Derbyshire (1963) stated that tissues and organs of healthy calves and piglets killed during the first 7 days of life harbored small numbers of bacteria in the lymph glands draining the head, intestines, and lungs, and large numbers in the tonsils. They also reported that the tonsils, the small intestine, and lungs were sites through which bacteria may enter the body. McCarthy et al. (1963) found that normal tissue of chicken harbored a small number of bacteria, both aerobic and anaerobic, ranging from 10 to 200 organisms per gram of tissue.

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Bruising or tissue injury consists of a number of independent events that lead to the localization and ultimate disposal of the injured tissue through healing. Studies in our laboratory by McCarthy et al. (1963) revealed that 61% of bruised tissues secured from a commercial processing line and 74.2% obtained from experimentally inflicted poultry bruises harbored both aerobic and anaerobic bacteria. These organisms were found in experimentally inflicted bruises to increase in number at the early stages of healing (1 to 2 days), followed by a rapid decrease to the level of the control within 4 to 6 days. Age of bruise, environmental conditions (sanitation of batteries, temperature, and moisture), severity of the bruise, and the presence of hemoglobin and its degradation products were among factors affecting the microbial content and growth of microbes in bruised tissue. McCarthy et al. (1963) also established that 36% of the gram-positive cocci isolated from these damaged tissues belonged to the genus *Staphylococcus*. Tests for identification of these staphylococci included oxygen requirement, coagulase production, growth on Staphylococcus 110 media (7.5% NaCl), mannitol and glucose fermentation (aerobically and anaerobically), and hemolysis, and revealed that 48% of these cultures were *S. aureus* and the other, *S. epidermidis*.

The present communication describes a series of experiments designed to examine the sources of organisms found in bruised tissue and to ascertain their possible portals of entry.

MATERIALS AND METHODS

Bruising and sampling. Apparently normal, white Leghorn chickens [8 to 10 weeks old; 3 to 4 lb (1.36 to 1.8 kg)], kept in a constant temperature house (22 C), were used. The birds were maintained in batteries, and were offered standard rations (free from medications) and water *ad libitum*. In the experiments where the marker strain of *Staphylococcus* was to be established in the gut, 1% HCl was substituted for their drinking water for 2 to 5 days prior to the experiment. This procedure, a modification of that reported by Schaedler and Dubos (1962), was used to eliminate staphylococcus organisms from the gastrointestinal tract.

The standard technique previously described by Hamdy, May, and Powers (1961) was adopted to inflict the bruises on the pectoralis major muscle with three blows. Symmetrically located areas on the same or different birds, or both, served as controls (unbruised). At specific time intervals during healing, the birds were killed by exsanguination and were examined aseptically under controlled conditions. Plates containing the appropriate media were exposed for the same intervals of time required to collect the samples. The counts and identification of bacteria obtained on these plates were used to determine the extent of microbial contamination present during sampling. All equipment and instruments used for excision and collection of samples were presterilized.

The skin and feathers of both control and bruised birds were swabbed with 70% alcohol, and the skin over the area to be assayed was excised without contaminating the bruised or control muscles. The surfaces of these tissues were swabbed with 70% alcohol; the samples were immediately excised and were placed in a sterile container to cool the samples sufficiently to prevent further growth of the organisms present. These tissues were minced and thoroughly homogenized with physiological saline in a 1:10 dilution at a low temperature by means of a sterile, precooled Sorvall omnimixer. The homogenates were then subjected to standard bacteriological procedures for detection and enumeration of the test organisms.

Enrichment techniques were used to detect the presence of small numbers of organisms in tissue homogenates, various internal organs, and blood. In this technique, swabs or samples were incubated in nutrient broth or nutrient broth with 7.5% NaCl for 24 hr, and were streaked on plates containing the appropriate media; the plates were examined for the test organism after incubation for 48 hr.

Cultures. The organisms employed in this study included: *Escherichia coli* K-12, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Serratia marcescens*, and a marker strain (MS) of *Staphylococcus aureus* (coagulase-, deoxyribonuclease-positive and mannitol-negative; phage type of 52/52A/80). These cultures, with the exception of the latter, were maintained on Tryptone Glucose Extract (TGE) agar (Difco), whereas the *S. aureus* MS was kept on nutrient agar (Difco).

Preparation of test organisms. In the gut experiments, flasks of nutrient broth were inoculated with active cultures of the test organisms and were used after incubation for 18 to 24 hr at 37 C.

For the experiments on the air sac, the test cultures were grown in flasks of nutrient broth, incubated for 18 to 24 hr, harvested by centrifugation, washed three times, and resuspended in sterile saline to the desired concentration.

Recovery of test organisms. The appropriate dilutions of the enriched swab, blood, tissue sample, or dilutions of blood or tissue homogenates were plated out on the following (Difco) media: Violet Red Bile Agar for *E. coli*; enrichment in Selinite Broth followed by streaking on SS agar for *S. enteritidis*; TGE for *S. marcescens*; the agar medium of King, Ward, and Raney (1958) for *P. fluorescens*, with examination of the fluorescent pigmented colonies by means of a 3,600 A ultraviolet lamp after 3 days of incubation at 20 C; and Mannitol Salt Agar and deoxyribonuclease test agar for *S. aureus* MS. Upon recovery of the test organisms from the birds, morphological or physiological examinations, or both, were performed when necessary.

Terminology. Unless otherwise stated, the terms *S.*

aureus MS, test organism, and marker strain in the results and discussion sections refer to the marker strain of *Staphylococcus* (coagulase-, deoxyribonuclease-positive and mannitol-negative).

RESULTS

Sources of organisms found in bruised normal tissue. A group of 27 birds were injected in the pectoralis major muscle with 0.25 ml of a saline suspension of *S. aureus* MS containing 18×10^6 cells. At various time intervals after injection, birds were killed, and their tissues, at the site of injection, were examined quantitatively for the test organism. The results showed that the number of viable cells decreased rapidly on the first and second days, and continued to decrease until they were no longer detected in the tissue on days 6 to 7.

After the establishment of the ability of normal tissue to eliminate *S. aureus* MS rapidly after intramuscular injection, a second group of 60 birds were inoculated as described previously. Examination of six birds 3 hr, and again 3 days, after injection revealed the same trend of the tissues' capacity to combat invasion of *S. aureus* MS. On the third day after injection, the remaining 54 birds were breast-bruised at the site of treatment. On the first day after bruise infliction, a sharp increase in the number of *S. aureus* MS in the contused area was evident. Although this number decreased by the second day, the marker strain was recovered from bruised tissue on the seventh day postinjection (Fig. 1).

Various other sources implicated as possible means of contaminating the bruised tissue were also examined (Table 1). Various genera of bacteria (including *S. aureus* and *S. epidermidis*) identical to those isolated from bruised tissue by McCarthy et al. (1963) were also obtained from poultry feed, feathers, air sacs, skin of birds, gut, and fecal material. All of these samples except air sacs harbored a large number of bacteria; the air sacs contained a small number. No similar organisms were recovered from drinking water or air of the chicken house. Staphylococcal organisms identical to those in bruised tissue were the predominant organisms in most of the samples examined. The possibility that poultry feed could be a predominant source of organisms in bruised tissue led to investigation of the gut as a portal of entry.

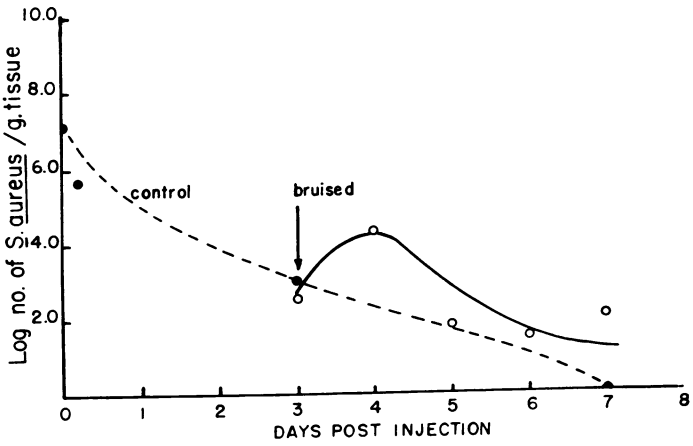

FIG. 1. *Normal tissue as a possible portal of entry. Effect of bruising on the fate of a small number of Staphylococcus aureus (marker strain) organisms in poultry tissue.*

The gut as possible portal of entry. First, *Staphylococcus* organisms were eliminated from the gastrointestinal tract of 126 birds to be used in these experiments by substituting a 1% HCl solution for the drinking water for a period of 2 to 5 days prior to feeding the test culture. Examination of their fecal material indicated the absence of all staphylococcus organisms. Then, a suspension of the marker strain in nutrient broth containing 10^6 to 10^8 cells per ml was administered to 105 birds either in place of their drinking water or directly by gavage. In the latter method, the suspension was administered directly into the gastrointestinal tract by means of an automatic syringe with a 4-in. (10.1 cm) 20-gauge canula covered by polyethylene tubing to prevent injury to the bird. The needle was inserted into the mouth and down through the esophagus, and 4 ml of the culture were administered to each bird twice daily. These feedings were continued throughout the course of the experiments to ensure the constant presence of the test organism in the gut. Fed-birds in which the marker strain was not recovered from the anus, gizzard, or intestinal tract prior to or at the time of exsanguination were not used in calculating the results reported herein.

After establishing the test organism in the gut of 105 birds (4 to 6 days after the feedings were begun), 63 birds were bruised; 42 served as control fed birds; and 21 represented control nonfed birds. Immediately after trauma and each day thereafter, bruised and an equal number of both controls (representing birds fed *S. aureus* MS and birds nonfed) were killed for cultures of their tissues, blood, and various organs.

It should be pointed out that neither pathological examinations nor leukocyte counts were performed on any of the experimental birds; therefore, the following

TABLE 1. *Sources examined for bacteria identical to those found in poultry tissues*

Source	*Staphylococcus**	Other genera
Drinking water of chickens	Absent	Absent
Air in poultry house	Absent	Absent
Feathers	Present	Present
Skin of birds	Present	Present
Air sacs	Present	Present
Poultry feed (McCarthy et al., 1963)	Present	Present
Gut of birds	Present	Present
Chicken droppings	Present	Present

* *Staphylococcus* was the predominant organism in bruised tissue (McCarthy et al., 1963).

interpretation of the results is based solely on the bacteriological analysis.

Examination of the bruised tissue revealed the presence of the test organism in small numbers from 0 to 5 days postcontusion. The incidence of positive culture for *S. aureus* MS in these tissues correlated with the age of bruise (Fig. 2). Immediately after bruising (0 to 1 day after bruise), 22% of the bruised tissues harbored the test organism. A maximum of 33% of the tissues contained the test organism on the 2nd day after bruising; thereafter, the marker strain was recovered from 22, 11, 11, and 0% of these tissues on the 3rd, 4th, 5th, and 6th days, respectively. On the other hand, the only incidence of positive culture in the tissues of control fed birds was noted at zero time and was 16%. Comparison of the total percentage of recoverable *S. aureus* MS in fed birds revealed that 17% of all bruised tissues and 2.5% of all control tissues harbored the test organism (Table 2).

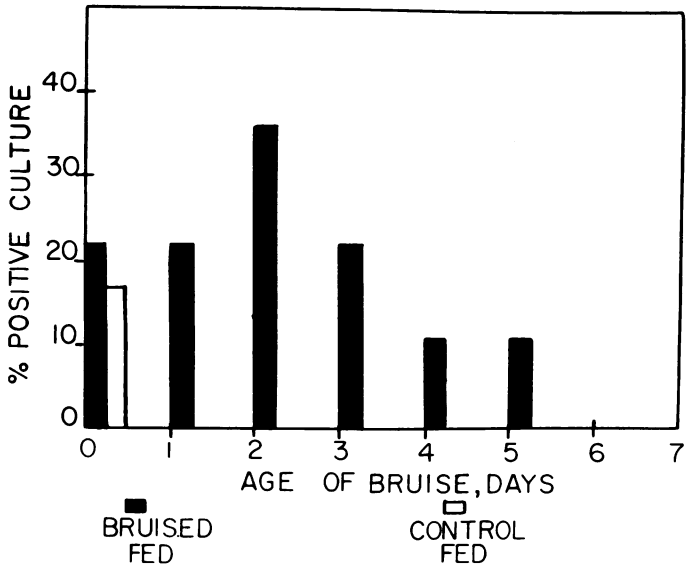

FIG. 2. *Per cent positive culture for Staphylococcus aureus (marker strain) in tissues of bruised fed and control fed birds as related to the age of the bruise.*

TABLE 2. *Incidence of positive cultures for Staphylococcus aureus, marker strain, in tissue, blood, serosa, and longitudinal muscles of the gut in bruised and control fed birds*

Source of culture	Days after bruise	Bruised birds		Control birds	
		No. cultured	Per cent	No. cultured	Per cent
Serosa and longitudinal muscles of the gut	0–1	16	43	11	36
	2–3	14	36	10	20
	4–5	16	12.5	11	36
	6	9	11	6	0
Total		55	27	38	26
Blood	0–1	16	12.5	11	18.2
	2–3	14	21	10	40
	4–5	16	25	11	45.5
	6	9	11	6	0
Total		55	18	38	29
Tissue	0–1	18	22	12	8
	2–3	18	28	12	0
	4–5	18	11	12	0
	6	9	0	6	0
Total		63	17	42	2.5

Among the control and bruised fed birds, slightly more than one-fourth of each group were found to have the test organism in the serosa or longitudinal muscles, or both, of their gut. Although migration of the test organism through the intestinal mucosa of the control fed birds appeared to occur at most times in a small percentage of the samples tested, bruising appeared to increase this migration initially, followed by a gradual decrease as a function of time postbruising (Table 2).

The attempt to demonstrate the test organism in the blood was successful in only 18% of all bruised fed and 29% of all control fed birds. However, no discernible pattern in the frequency of the test organisms isolated from the circulatory system was noted in either group of birds.

Analysis of the tissue, blood, serosa, and longitudinal muscles of the gut of control nonfed birds revealed the absence of the test organism at all times.

This experiment was repeated with the following cultures: *E. coli* K-12, *P. fluorescens*, *S. marcescens*, and *S. enteritidis*. However, recovery of these organisms except *S. enteritidis* was not attained from the bruised or control tissue, in spite of repeated isolation of these bacteria from the intestinal tract. When *S. enteritidis* was used, the incidence of positive culture in the tissue of the fed birds at the early stages of healing (0 to 1 day after bruise) was 6% in the bruised and 0% in the control.

Air sac. Cultures of the intraclavicular, anterior thoracic, and posterior thoracic air sacs revealed the presence of various types of bacteria (Table 3), confirming the

TABLE 3. *Frequency of isolation of Staphylococcus aureus and other groups of bacteria from various air sacs of 22 normal birds*

Genera and groups of bacteria	Anatomical division of air sacs*					
	Right			Left		
	IC	AT	PT	IC	AT	PT
S. aureus	2†	2	2	2	0	1
Other *Staphylococcus* spp.	9	12	12	11	14	12
Lactobacillus	1	0	1	1	0	1
Yeast	3	2	8	9	7	8
Micrococcus spp.	2	2	1	3	2	1
Long and short rods	1	2	1	1	2	1
Miscellaneous	3	0	1	5	3	3
No growth	7	7	5	4	4	4

* Abbreviations: IC, intraclavicular air sac; AT, anterior thoracic air sac; PT, posterior thoracic air sac.

† Number of birds from which organisms were recovered.

results of Van Roekel et al. (1957) and Smibert, Faber, and DeVolt (1960). Therefore, an experiment was conducted to investigate the role that might be played by the air sac as a site of entry of bacteria found in bruised tissue.

The left intraclavicular air sac of 24 birds was injected through the shoulder muscle with 0.20 ml of broth culture containing 50×10^6 cells of *S. aureus* MS. At 2 days postinjection, eight birds were breast-bruised on the left side (group A), eight birds were bruised on both sides of the breast (group B), and eight birds served as unbruised controls (group C). At various time intervals during healing, birds from each group were sacrificed, and the bruised and control tissues, blood, liver, and air sacs were examined for the presence of the test organism. The results of this experiment revealed that *S. aureus* MS, established in the air sac, was recovered from 17% of bruised tissues secured from birds of group A and B at the early stages of healing (1 to 2 days after bruise infliction), but not from either the nonbruised tissues of group A or the control tissues obtained from birds of group C, indicating that the air sac may also serve as a possible portal of entry.

The validity of this experiment was doubtful, owing to the possibility that the test organism may have been introduced into the muscle during the injection of *S. aureus* MS into the intraclavicular air sac. Therefore, this experiment was repeated, except for inoculating the respiratory system intratracheally with 1 ml of culture containing 1.5×10^9 cells. The inoculum was introduced into the lower one-third of the trachea by means of a 4-in. (10.1 cm) 20-gauge canula. At 2.5 hr postinjection, 28 birds were breast-bruised on the right pectoralis major muscle (group A); 12 birds served as inoculated controls (group B); and 22 birds served as unbruised, uninoculated controls (group C). Again, at various time intervals during healing, birds from each group were killed, and bruised and control tissues and all the air sacs were examined for the presence of the test organism. The results of this experiment revealed the absence of the test organism at all times in the air sacs, lung, and tissue of the uninoculated birds (group C), as well as in the tissue of the control inoculated birds (group B). However, the test organism was recovered from 25% of the air sacs and 75% of the lungs of both the control and the bruised inoculated birds (Table 4). Of the 28 bruised, intratracheally inoculated birds, the positive incidence for the test organism was noted in only one tissue immediately after trauma.

TABLE 4. *Frequency of isolation of Staphylococcus aureus, marker strain, from air sacs and other tissues of 28 bruised and 12 control (unbruised) birds injected intratracheally*

Determination	Days post-injection	Anatomical division of air sac*						Lung	Muscle
		Right			Left				
		IC	AT	PT	IC	AT	PT*		
Bruised.....	0	14†	72	43	0	57	14	100	14
	1	29	43	57	0	29	14	72	0
	2	0	0	29	0	14	0	43	0
	3	0	0	0	0	0	0	57	0
Control.....	0	66	100	100	66	33	0	100	0
	1	0	33	66	33	33	33	66	0
	2	0	33	33	33	33	33	33	0
	3	0	33	33	0	33	33	66	0

* Abbreviations: IC, intraclavicular air sac; AT, anterior thoracic air sac; PT, posterior thoracic air sac.

† Percentage of birds from which the marker strain was recovered.

DISCUSSION

The quantitative bacteriological examinations reported here on the possible sources of organisms, including staphylococcus, found in poultry bruises, indicated that many different sources (normal tissue, air sacs, feathers, skin of birds, gut, and poultry feed) harbored various types of bacteria that were identical to those isolated from poultry bruises. Further investigation of normal tissue revealed that, after the intramuscular injection of *S. aureus* MS, the defense mechanism, demonstrated by Smith and Dubos (1956) in normal animals, eliminated the test organism within 7 days. The act of bruising birds on the third day after injection increased the number of the test organism recovered 1 to 4 days postcontusion. It is felt, therefore, that normal tissue, when traumatized, may serve as a source of bacteria detectable in poultry bruises.

The present study confirmed and extended previous work (McCarthy et al., 1963) which established poultry feed as an important source of these organisms. Therefore, a two-step procedure was employed to examine the assumption that the gut might serve as a portal of entry. (i) A 1% hydrochloric acid solution was substituted for the drinking water of birds for 3 to 5 days prior to bruising to eliminate staphylococcus organisms from the gastrointestinal tract. (ii) A test organism was established in the gut of these birds through continuous feedings with an active culture prior to and during the entire experiment.

It should be pointed out that the hydrochloric acid sterilization of the gut prior to feedings of the test organisms may have interfered with the normal physiology of the gut. No studies were performed to determine the extent of trauma caused by this procedure. If this sterilization technique affected the permeability of the gut and facilitated the migration of the test organisms through the intestinal mucosa, the results obtained from both control and bruised birds should be the same.

It is plausible that organisms used in this investigation penetrated the intestinal mucosa continually in small amounts in both control and bruised birds. Examination of the serosa and longitudinal muscles of the gut revealed

that 25% of both groups of birds allowed the test organism to traverse this barrier. However, an increase in the migration of two of the test organisms (*S. aureus* and *S. enteritidis*) was observed initially after bruising. Since the other organisms used apparently did not traverse the intestinal barrier, the hypothesis that the gut may serve as a portal of entry of bacteria to traumatized areas cannot be ignored.

The results obtained demonstrated that both *S. entertidis* and virulent *S. aureus* MS were able to contaminate the tissues of bruised birds. The incidence of positive culture of these test organisms in the tissues correlated with the age of bruise, being greatest on the 1st and 2nd days. However, the incidence of positive *S. aureus* MS was much higher than that of *Salmonella* recovered from tissues of bruised birds fed the test organisms. The failure to recover *E. coli*, *P. fluorescens*, and *S. marcescens* from bruised tissue may be due to the lack of invasiveness of these organisms as compared with the more virulent bacteria.

Although in only one incidence were any of the test organisms recovered from control tissues, it is possible that their number in these areas was too small to detect. The presence of a broad spectrum of hydrolytic enzymes in experimentally inflicted poultry bruises (Hamdy et al., 1961; Brown and Hamdy, 1964; and Brown, 1964) is believed to alter the permeability of the tissue at the site of the bruise and facilitate the invasion and the growth of bacteria accordingly. Once small numbers of these bacteria reached the bruised areas, the presence of these hydrolytic enzymes, fluid, extrastromal hemoglobin (Hamdy et al., 1961), and the degradation product of deoxyribonucleic acid (Brown, 1964) in the contused tissues may have stimulated their growth.

Examination of the air sacs revealed the presence of various types of bacteria identical to those found in bruised tissue. These results pointed out that air sacs may be another site by which organisms invade bruised areas. After the injection of a test organism in the air sac, it was recovered from 75% of the lungs and 25% of the air sacs in all bruised and control injected birds. Although the test organism was recovered from a greater percentage of control injected air sacs, the only incidence of the test organism in the tissue was in the bruised injected birds. The results obtained from this experiment supported the previous assumption that the air sac may serve as a possible site of entry of bacteria to the bruised tissue, but to a limited extent.

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